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Reproductive cycle of *Microtus pyrenaicus* De Sélys-Longchamps, 1847 (Mammalia, Rodentia) in the Western Pyrenees

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Abstract

Studied was the reproductive cycle of *Microtus pyrenaicus* in the Quinto Real Mountains (Western Pyrenees, Iberian Peninsula). 131 specimens (61 males, 70 females) were analyzed. Results indicate the presence of sexually active males throughout the year, ranging from 25% in winter to 100% in spring. Females have a similar cycle, although in December, January and March no sexually active female was found and in February only 20% showed signs of activity. The female breeding season is April–November, with a significant decline from December to March. From June to September all mature females are sexually active, and 67.9% of females were pregnant. The average litter size was 2.5 (se = 0.15; 1–3; n = 18) and the average of the placental scars was 2.4 (se = 0.15; 2–3; n = 11). In comparison with other voles closely related phylogenetically, *Microtus pyrenaicus* has a lower average litter size than cited by Krapp (1982) for *Microtus savii*. The winter break in reproductive activity suggests a relation to the annual cycle of food availability (prairie grasses) or perhaps to the harsh climate endured.

Introduction

The Pyrenean vole, *Microtus pyrenaicus* occupies areas in the Iberian Peninsula and France with the southern limits in the Pyrenees (Niethammer 1956, 1964; Castién and Mendiola 1984; Gosálbez 1987) and northern limits in Central France (Spitz 1984; Krapp 1982).

Information on its reproductive cycle is scarce. Only Saint-Girons (1973) provides data on litter sizes. The purpose of this study is to define the most relevant characteristics of the reproductive cycle of this species in the wild.

Materials and methods

The 131 specimens analysed (61 males, 70 females) were captured between September 1984 and August 1986 in the Quinto Real mountains (Western Pyrenees, northern Iberian Peninsula) at altitudes of between 660 and 850 m. Average annual rainfall in the study area was 2.138 l/m^2. Maximum rainfall occurred in April (243.5 l/m^2) and November (253.2 l/m^2) and the minimum in July (68.4 l/m^2). Temperatures were at minimum in January (= 2.9 °C) and maximum in August (= 16.6 °C). The average annual temperature was 8.8 °C. Most animals were captured in small clearings in the forest, except for three specimens from shrubland and river banks.

In each specimen the external features relevant to reproduction were noted: in males, the position of testicles (abdominal or scrotal), in females, vaginal perforation, presence of sperm plugs or symptoms of weaning, as shown by size and development of teats. In males the length of the seminal vesicle and the maximum orthogonal length of each testicle were measured. A smear of testicular material and epi-

didymis was also carried out. The cell content of this smear was stained according to the criteria mentioned by Gosálbez et al. (1979). In females the reproductive apparatus was extirpated and characteristics of irrigation and dilatation of the uterus were noted as symptoms of reproductive activity. In pregnant females the number of embryos in each uterus was noted. The number of recent placental scars was taken, and these were differentiated from older ones.

The samples were classified according to the criteria of Ventura and Gosálbez (1987). In males three categories have been established depending on sexual maturity. Immature: specimens with no spermatid or spermatozoa in the testicles. Submature: animals with few spermatids or spermatozoa in the testicles. Mature: animals with a large amount of spermatozoa in the testicles. The observations of the epididymis supported this classification and, in some cases, provided complementary information.

In females the following categories were established: Immature: closed vulva, poorly developed uterus, lack of placental scars. Inactive mature: closed vulva, partly developed uterus but scantily vascularized. These may present placental scars but not embryos. Active mature: open vulva, fully developed and well vascularized uterus. They may present embryos or placental scars.

Results

Reproduction in males

Figure 1 shows the relation between the product of the major testicular diameters and the length of the seminal vesicle, differentiating the sexual stage of each specimen. Immature animals showed values of less then 7.6 mm in the length of the seminal vesicle and 29.8 mm^2 in the product of the major testicular diameters. Submature males had a wide range (seminal vesicle length from 1.5 mm to 11.5 mm and the product of the major testicular diameters ranges from 14.3 mm^2 to 35.7 mm^2). Mature animals had a seminal vesicle over 6 mm long and a product of the major testicular diameters over 12.0 mm^2. The highest value found in seminal vesicle length was 14.5 mm and for the product of the major testicular diameters 79.6 mm^2.

Data derived from the study of spermatozoid presence in the testicle and epididymis indicate that, in animals with seminal vesicle between 6.0 mm and 11.4 mm, there are spe-

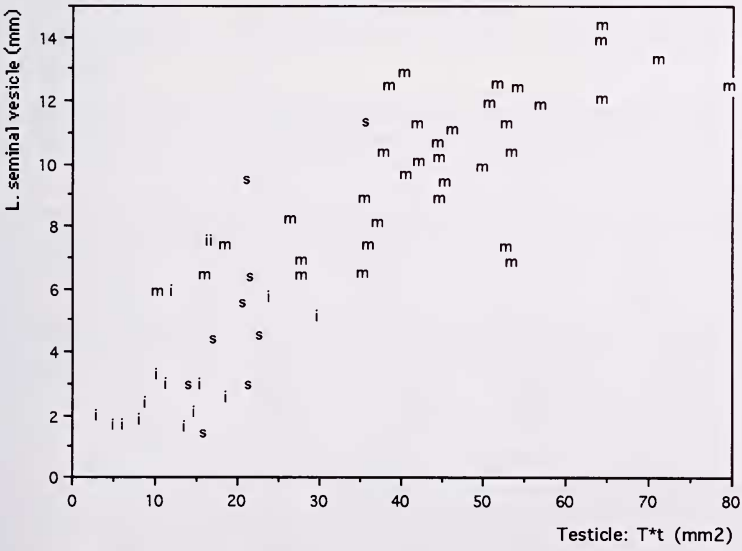

Fig. 1. Relationship between the length of the seminal vesicle and the product of the major testicular diameters, differentiating the sexual maturity. i: immature, s: submature and m: mature. n = 60.

cimens with no trace of spermatozoa and others that have initiated spermatogenic activity. Specimens with seminal vesicle length over 11.4 mm all show the presence of spermatozoa in the gonad. Several low weight specimens are active, while all animals over 21.3 g are sexually active (the lightest sexually active animal found was captured in January 1985; its body mass was 11.8 g).

The variation in the average testicle length throughout the year (Fig. 2) present a variation with a significant decrease during the winter months, especially in January and an increase from spring onwards, registering maximum values in May and August with another marked decline in activity during July. In interpreting this variation, it should be taken into consideration that in July immature and submature specimens from the first litters of the year are included, thus causing a decline in the dimensions of the parameters commented on. These variations indicate a tendency towards a seasonal biological cycle, although in the case of males this tendency is dominated by the constant presence of sexually active animals (Tab. 1). The presence of high vesicular lengths in December may by due to an artefact of the sample.

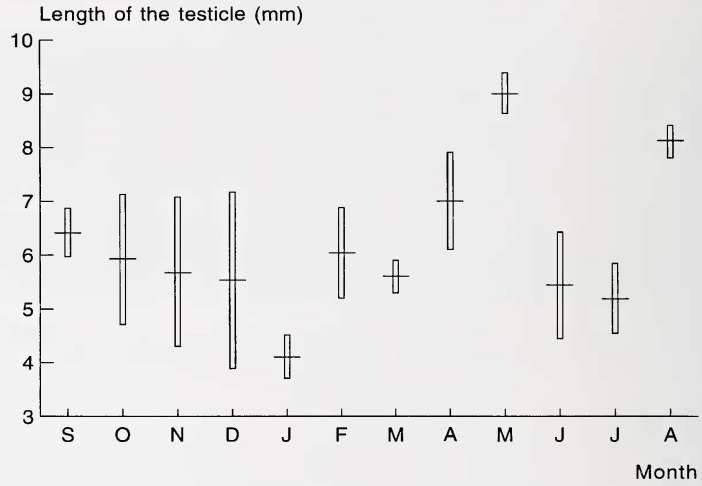

Fig. 2. Variation of the average testicular length (mm) with their standard error, throughout the study period. n = 61.

Table 1. Number and percentage of males of *M. pyrenaicus* distributed by months and reproductive categories.

	Sp		Oc		Nv		Dc		Jn		Fb	
	%	n	%	n	%	n	%	n	%	n	%	n
Immatures	16.6	1	25.0	1	75.0	3	33.3	1	75.0	3	40.0	2
Submatures	16.6	1	25.0	1	0.0	0	0.0	0	0.0	0	20.0	1
Matures	66.7	4	50.0	2	25.0	1	66.7	2	25.0	1	40.0	2

	Mz		Ap		Mi		Jn		Jl		Ag	
	%	n	%	n	%	n	%	n	%	n	%	n
Immatures	11.1	1	0.0	0	0.0	0	33.3	2	60.0	3	0.0	0
Submatures	44.4	4	0.0	0	0.0	0	16.7	1	20.0	1	0.0	0
Matures	44.4	4	100.0	4	100.0	5	50.0	3	20.0	1	100.0	6

Table 1 shows the proportion and number of reproductive classes for each month of the year. The presence of mature specimens throughout the year should be noted. After September there is an increase in the number of immature animals, which reaches maximum between November and January. From this month on there is a progressive increase in the number of mature animals, due to the acquisition of sexual maturity by specimens born at the end of the previous year. This is demonstrated by the capture of submature specimens in subsequent months, until April, when 100% of male captures were mature. After June immature specimens began to appear again, which, on the basis of their weight, correspond to the first litters of the year. These animals were soon incorporated into the group of reproducers, as can be seen from the simultaneous presence of submature animals.

Reproduction in females

All females weighing less than 14.5 g were immature. Mature, inactive and active specimens ranged from 14.5 g to 24.2 g. The heaviest female captured without sign of sexual activity weighed 19.6 g.

Table 2 represents the proportion and number of females in each reproductive category throughout the year. The results show that from September onwards the number of immature specimens increased progressively due to delayed sexual maturity in winter. During December and January no sexually active specimen was captured. From then on there was a progressive incorporation of active animals, so that between June and September all mature females were capable of reproduction. Pregnant females were found between April and November (Tab. 2).

The average litter size, calculated from embryo counts, is $\bar{x} = 2.5$ (se = 0.15; 1–3; n = 19). The resulting value of placental scar counts is $\bar{x} = 2.4$ (se = 0.15; 2–3; n = 11). It is worth noting that average litter sizes in summer ($\bar{x} = 2.14$, n = 7, se = 0.26) were slightly lower than those in spring ($\bar{x} = 2.67$, n = 6, se = 0.21) and autumn ($\bar{x} = 2.80$, n = 5, se = 0.20), although the comparison of averages (Student's t test) does not show any significant difference, (summer – spring: t = 1.525; p = 0.155, summer – autumn: t = 1.856; p = 0.093). The percentage of pregnant females compared with active mature specimens in the period June–September is 67.9%.

Table 2. Number and percentage of females distributed by months and reproductive categories. Number and percentage of sexually active *M. pyrenaicus* pregnant each month related to matured females.

	Sp		Oc		Nv		Dc		Jn		Fb	
	%	n	%	n	%	n	%	n	%	n	%	n
Immatures	0.0	0	33.3	2	25.0	1	100.0	2	75.0	3	40.0	2
Inactive matures	0.0	0	50.0	3	25.0	1	0.0	0	25.0	1	40.0	2
Active matures	100.0	2	16.7	1	50.0	2	0.0	0	0.0	0	20.0	1
Pregnant matures	100.0	2	25.0	1	66.7	2	0.0	0	0.0	0	0.0	0

	Mz		Ap		Mi		Jn		Jl		Ag	
	%	n	%	n	%	n	%	n	%	n	%	n
Immatures	33.3	2	50.0	5	14.3	1	64.3	9	0.0	0	33.3	1
Inactive matures	66.7	4	0.0	0	14.3	1	0.0	0	0.0	0	0.0	0
Active matures	0.0	0	50.0	5	71.4	3	35.7	5	100.0	6	66.7	2
Pregnant matures	0.0	0	60.0	3	50.0	3	100.0	5	50.0	3	0.0	0

Sex ratio

Table 3 shows the sex ratio in each season through the chi-square test. In autumn and winter the proportions are similar, while in spring and summer there are more females. The value of chi-square indicates that there is no significant variation on the expected value 1 : 1. The same result is applicable to the total number of captures.

Table 3. Number and proportion of sexes obtained in each season of the year.

	Autumn		Winter		Spring		Summer		Total	
	n	%	n	%	n	%	n	%	n	%
Females	12	(46.2)	12	(50.0)	23	(56.1)	23	(57.5)	70	(53.4)
Males	14	(53.8)	12	(50.0)	18	(43.9)	17	(42.5)	61	(46.6)
Chi-Squ.	0.154		0.000		0.610		0.900		0.618	
Deg. fr.	1		1		1		1		1	
p	0.695		1.000		0.453		0.343		0.431	

Discussion

There are few reports in the literature on the biological cycle of *Microtus pyrenaicus*. In relation to litter size, Saint-Girons (1973) mentions the capture of 3 active females in the Pyrenees, one with four placental scars, another with two and a third pregnant with one embryo. Those figures concord with ours, though in the study population no animal was found with 4 embryos.

In comparison with other voles closely related phylogenetically, Krapp (1982) states that the average litter size in *Microtus savii* is 3.3 (2–4, $n = 9$) which is larger than the average obtained for *Microtus pyrenaicus* in this study ($t = 5.485$; $p < 0.01$).

The winter break in reproductive activity suggest a relation to the annual cycle of food availability (prairie grasses) or perhaps to the harsh climate endured by these nuclei of Pyrenean mountain voles.

The sex ratio shown is similar to that found in other European Microtinae (Winking 1976; Palomo et al. 1989; Kratochvíl 1969).

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Zusammenfassung

Fortpflanzungszyklus von Microtus pyrenaicus de Sélys-Longchamps, 1847 (Mammalia Rodentia) in den Westpyrenäen

Es wird über den jährlichen Fortpflanzungszyklus der Pyrenäen-Kleinwühlmaus (*Microtus pyrenaicus*) im Quinto Real Gebirge (Westpyrenäen, Iberische Halbinsel) berichtet. Es wurden insgesamt 131 gefangene Exemplare (61 Männchen, 70 Weibchen) ausgewertet. Im untersuchten Gebiet treten geschlechtsreife Männchen in allen Monaten auf. Jedoch schwankt ihr Prozentsatz in bezug auf die Gesamtanzahl der Männchen bemerkenswert im Jahreslauf. Der niedrigste Wert wird im Winter erreicht (25%), der höchste im Frühjahr (100%). Die Fortpflanzungsperiode der Weibchen beginnt offenbar im April und kann bis Ende November dauern. Jedoch prägt sich die Fortpflanzungsintensität von

Juni bis September am stärksten aus. In diesem Zeitraum sind alle geschlechtsreifen Weibchen fortpflanzungsaktiv, die meisten (67,9%) sogar trächtig. Von Dezember bis Ende März nimmt die Geschlechtstätigkeit der Weibchen ab. Der Gesamtmittelwert der Embryonen je Wurf und der Placentanarben je Weibchen beträgt jeweils 2,5 ± 0,15 (1–3, n = 18) und 2,4 ± 0,15 (2–3, n = 11).

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